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Impact of pesticide application on zooplankton communities with different densities of invertebrate predators: An experimental analysis using small-scale mesocosms

K.H. Chang*, M. Sakamoto, T. Hanazato

Research and Education Center for Inlandwater Environment, Shinshu University, Kogandori 5-2-4, Suwa 392-0027, Japan Received 27 July 2004; received in revised form 28 January 2005; accepted 1 February 2005

Abstract

We assessed the responses of zooplankton communities with different population densities of an invertebrate predator, Meso-cyclops pehpeiensis, to insecticide (carbaryl, 0.5 mg L^{-1}) in small-scale mesocosm tanks (20 L). Cladocerans were eliminated by carbaryl application at both high and low predator densities. The density of rotifers increased after the elimination of the cladocerans by carbaryl application at low-predator density but not at high-predator density. Carbaryl application increased the relative importance of predatory interactions in the zooplankton community. The results suggest that predator abundance can affect the response of a zooplankton community to carbaryl application through predation on surviving zooplankton. © 2005 Elsevier B.V. All rights reserved.

Keywords: Pesticide; Zooplankton; Mesocosm; Food web; Predation

1. Introduction

Zooplankton are important organisms in freshwater ecosystem since they occupy a central position in the food chain. They transfer energy from primary producers to higher trophic organisms such as fish, and their community structure, biomass, and production influence the whole food web structure of freshwater ecosystems through trophic interactions (Mills and Forney, 1988). At the same time, they are one of the

E-mail address: changkwang38@hotmail.com (K.H. Chang).

groups most sensitive to toxic chemicals (Hanazato, 2001). Thus, they have been frequently used in ecotoxicological tests (OECD, 1981; Japanese Society of Environmental Toxicology, 2003). Among many toxic chemicals, pesticides affect zooplankton at individual, population, and community levels (Goodrich and Leach, 1990; Dodson et al., 1995; Hanazato, 1998a, 2001).

Recent ecotoxicological studies have concentrated on the community level responses of zooplankton to contamination by toxic chemicals, including pesticides (Hanazato and Kasai, 1995; Sierszen and Lozano, 1998; Lahr et al., 2000; Kreutzweiser et al., 2002), in relation to the zooplankton's functional roles in fresh-

^{*} Corresponding author. Tel.: +81 266 52 1955; fax: +81 266 57 1341.

water ecosystems. As a community, zooplankton include many different species at different trophic levels in the food web. The application of toxicants can differentially alter the population densities of predators and prey, and affect prey-predator interactions in the community (Hanazato, 1998b; Preston et al., 1999a,b). Since predation has an important impact on zooplankton populations through top-down regulation, the impact of toxicants on a zooplankton community can be seriously affected by the presence of predators through changes in predator-prey interactions in the community. The influence of Chaoborus larvae (Diptera, Chaoboridae) and a predacious rotifer, Asplanchna, on the impact of insecticide application on a zooplankton community has been studied in mesocosms such as artificial ponds and enclosures (Hanazato, 1991; Peither et al., 1996). It may also be true that the presence of cyclopoid copepods, common invertebrate predators in freshwater, can influence the effects of insecticides on zooplankton communities. However, the relationship between the presence of copepods and insecticides in their effects on zooplankton communities is poorly understood, probably owing to the difficulties in manipulating the density of copepods in mesocosms.

We compared the response of zooplankton communities with different population densities of the predacious copepod *Mesocyclops pehpeiensis* to pesticide application in mesocosms. Mesocosms such as experimental ponds and enclosures are frequently used as model systems to examine the response of zooplankton to chemical application at a community level. However, their size often causes difficulties in controlling experimental environments. In particular, it is difficult to control invertebrate predators such as copepods, which often develop high population density in large mesocosms. To exclude these problems, we used small-scale mesocosms (20 L), in which we could control temperature, food condition, and predation pressure by the copepods.

2. Methods

2.1. Model ecosystem

The experiment was set up on 19 April 2003 (day 0) and terminated on 8 June 2003 (day 46). Twelve

20-L cylindrical polyethylene tanks (diameter, 30 cm; height, 31 cm) were used as the mesocosms. The tanks were lined with polyethylene film to avoid any influence of previous experiments. To establish the zooplankton communities, 1 kg of bottom mud from the eutrophic Lake Suwa (36°2′N, 138°5′E), Japan, containing resting stages of zooplankton was placed in each tank on day 0. The bottom mud was collected with an Ekman-Birge dredge from the lake on 9 April 2003, and was stored in a refrigerator (4 °C) until the experiment was set up. All the tanks were kept in a temperaturecontrolled room (20 °C) with a photoperiod of 16 h light and 8 h dark. The green alga Chlorella (Chlorella Industry Co. Ltd., Fukuoka, Japan) was added to the tanks to a final density of approximately 3.3×10^4 cells mL^{-1} on day 10 and every 3 days thereafter.

The experimental procedure is illustrated in Fig. 1. The tanks were divided into two groups: high and low predator (M. pehpeiensis) densities. Since some cyclopoid copepods emerged from the resting stage in the bottom sediment, maintaining the complete absence of the predators in the tanks was impossible. We increased the density of M. pehpeiensis by introducing 40 late copepodites or adults into each high-predator-density tank on day 24. The introduced M. pehpeiensis came from tanks prepared as a Mesocyclops pool. Those tanks included bottom mud of Lake Suwa and were maintained for more than 2 months with high Chlorella density and high densities of rotifers and small cladocerans, the food of M. pehpeiensis. In contrast, adult M. pehpeiensis in the low-predator-density tanks were caught using a pipette on day 24. To minimize disturbance to other zooplankton in the tanks, we caught M. pehpeiensis near the surface quickly and gently. On day 33, 10 mg of carbaryl (Wako Pure Chemical Industries Ltd., Japan) diluted with 50 mL of solvent (ethanol) was added to the tanks to produce a nominal concentration of 0.5 mg L^{-1} . Solvent only (50 mL)was added to half of the tanks with each predator density as controls (Fig. 1). The basic environmental factors in the tanks during the experiment are summarized in Table 1 and show little variance between the tanks.

2.2. Zooplankton sampling and analysis

Before the carbaryl application (day 33), samples were collected on days 12, 19, 24, and 33. After the

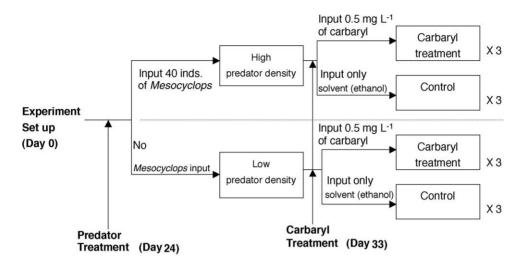


Fig. 1. Experimental procedure. The experiment was started on 19 April 2003 (day 0) and was terminated on 8 June 2003 (day 46).

carbaryl application, samples were collected on day 34 (24 h after application) and every 3 days thereafter. Zooplankton were sampled from each mesocosm in acrylic tube sampler (diameter, 5 cm; length, 62 cm) which has rubber caps at the top and bottom. The sampler was inserted vertically into the tank to a depth of 25.5 cm from the surface, and then the top and bottom were sealed to enclose 500 mL of the tank water. Two samples totalling 1 L were collected. Since zooplankton often aggregated near the wall of the tank, the water was gently mixed before the sampling. Collected water was filtered through a 40-µm mesh net and preserved with sugar-formalin at a final concentration of 4% (Haney and Hall, 1973). The fixed samples were concentrated to 5 mL by settling for 24 h. Aliquots of 1 mL were used for counting rotifers and copepod nauplii. Whole samples were used for counting cladocerans and copepodids.

To assess the impact of carbaryl on zooplankton community structure, a food web was drawn for each

tank on each sampling date, and the interactions of zooplankton in the food web were analyzed as follows (Sprules and Bowerman, 1988; Locke and Sprules, 1994; Kreutzweiser et al., 2004):

- (1) Number of trophic interactions per species: all trophic interactions between species, including cannibalism and mutual predation, were counted and divided by total number of species.
- (2) Number of predatory interactions: one trophic link was counted for each predator-prey species interaction. Cycles and mutual predation were also counted.
- (3) Number of competitive interactions: one potential competitive link was counted for each pair of species sharing food items. Competing species that were herbivorous at any ontogenetic stage were considered to share a phytoplankton resource.
- (4) Relative importance of predation: relative importance measures the proportions of predatory inter-

Average (± S.E.) water temperature, dissolved oxygen concentration (DO), and pH during the experimental period

	Water temperature (°C)	$DO (mg L^{-1})$	pН
Low-predator density			
Control	20.28 ± 0.03	5.65 ± 0.48	6.70 ± 0.06
Carbaryl treatment	20.38 ± 0.03	4.85 ± 0.62	6.69 ± 0.05
High-predator density			
Control	20.25 ± 0.04	5.68 ± 0.46	6.64 ± 0.07
Carbaryl treatment	20.43 ± 0.03	5.16 ± 0.57	6.68 ± 0.06

actions in the food web, and is calculated as follows:

Relative importance of predation = (number of predatory interactions)/(total interactions, including predatory and competitive interactions).

The differences in all quantitative data were tested with repeated-measures ANOVA using StatView ver. 5 (SAS Institute Inc., Cary, NC, USA).

3. Results

Two genera of predacious cyclopoid copepod, *M. pehpeiensis* and *Thermocyclops* sp., lived in the mesocosm tanks. Copepodids and adults were classified into genera, and their naupliar instars were classified as nauplii. After day 24, when the predator numbers were adjusted, the density of *M. pehpeiensis* was

higher in the high-predator-density tanks, as expected (Fig. 2), but the density of *Thermocyclops* remained low (<2 ind. L⁻¹) in all tanks and showed no marked differences among the tanks. No serious decrease of *M. pehpeiensis* density occurred after carbaryl application. The density of copepod nauplii was much higher in the control high-predator-density tanks. In carbaryl-treated tanks, their density decreased after carbaryl application and remained significantly lower than in control tanks in both the high-predator-density tanks (RM-ANOVA time \times treatment interaction, F(1, 28) = 11.217, P < 0.005) and the low-predator-density tanks (RM-ANOVA time \times treatment interaction, F(1, 28) = 14.124, P < 0.001).

Ceriodaphnia quadrangula and Bosmina longirostris were the dominant cladoceran species in the tanks. The density of total cladocerans was much lower in the high-predator-density tanks (Fig. 3). On the other hand, they were eliminated by carbaryl application, and

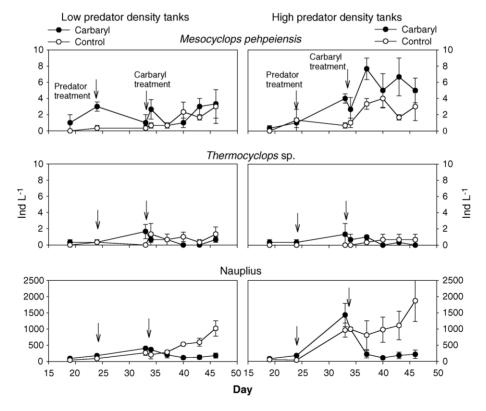


Fig. 2. Changes in the densities (average \pm S.E.) of copepodids and adults of *M. pehpeiensis* and *Thermocyclops* sp., and the copepod nauplii in the mesocosm tanks.

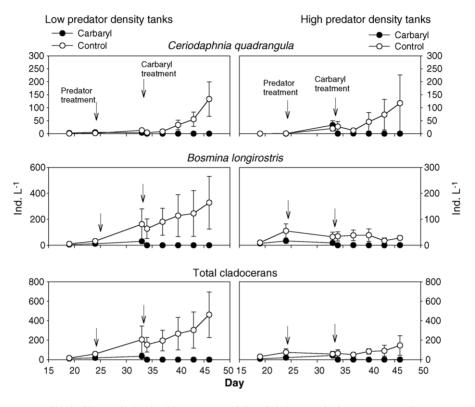


Fig. 3. Changes in the densities (average \pm S.E.) of cladocerans in the mesocosm tanks.

the population did not recover by the end of experiment. Trichocerca stylata, Hexarthra mira, and Lepadella sp. were the most abundant rotifer species. Their patterns of change of density after carbaryl application differed depending on predator density (Fig. 4). The densities of T. stylata were significantly higher in control tanks both at high- and low-predator density (RM-ANOVA time \times treatment interaction, F(1,(28) = 19.152, P = 0.0002 at low-predator density and F(1, 28) = 10.579, P = 0.003 at high-predator density). However, the density of T. stylata was much lower at high-predator density. The density of H. mira increased only in the control tanks at high-predator density, and showed significantly higher density than that in carbaryl tanks (RM-ANOVA time × treatment interaction, F(1, 28) = 5.057, P = 0.0326). Lepadella sp. increased its density after carbaryl application in the low-predator-density tanks, and showed much higher density than the tanks without carbaryl treatment (RM-ANOVA time \times treatment interaction, F(1,(28) = 5.798, P = 0.0229). However, such an increase

of Lepadella sp. density after carbaryl application was not observed in the high-predator-density tanks (RM-ANOVA time \times treatment interaction, F(1,(28) = 0.219, P = 0.6431). Consequently, the total density of rotifers increased after carbaryl application, and was higher in carbaryl-treated tanks than in control tanks at low-predator density with nearly significant level (RM-ANOVA time × treatment interaction, F(1, 28) = 3.098, P = 0.0893). However, rotifer density did not increase after carbaryl application in the highpredator-density tanks, and was rather higher in the control tanks, and showed opposite patterns to that in the low-predator-density tanks. Although the difference of total rotifer density between the control and carbaryl-treated tanks in high predator-densitytanks was not significant, but showed very low Pvalue (RM-ANOVA time \times treatment interaction, F(1,(28) = 2.795, P = 0.1057). Changes over time in densities of rotifers, cladocerans, M. pehpeiensis, and copepod nauplii in the treated tanks (predator introduction and/or carbaryl application) relative to those in the con-

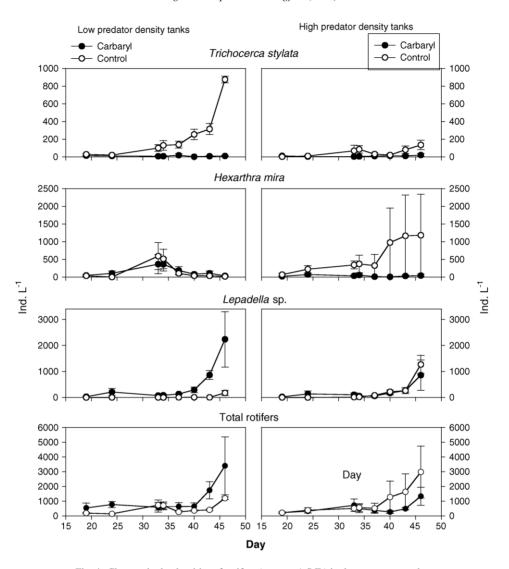


Fig. 4. Changes in the densities of rotifers (average $\pm\,\text{S.E.})$ in the mesocosm tanks.

trol tanks (no predator and no carbaryl) are shown in Fig. 5.

Despite the changes in densities and succession patterns of rotifers and nauplii after carbaryl application, the number of trophic interactions per species, which represents the food web structure and stability, was not affected by the carbaryl application (Fig. 6) at either low-predator density (RM-ANOVA time × treatment interaction, F(1, 28) = 0.456, P = 0.505) or high-predator density (RM-ANOVA time × treatment interaction, F(1, 28) = 0.687, P = 0.414). No remarkable

differences in numbers were found also between the low- and high-predator-density tanks. However, carbaryl application increased the relative importance of predatory interactions in the food web at both low-predator density (RM-ANOVA time \times treatment interaction, F(1, 28) = 3.311, P = 0.0677) and high-predator density (RM-ANOVA time \times treatment interaction, F(1, 28) = 3.542, P = 0.0703) with near significant level. The higher relative importance of predatory interactions was greater at high-predator density, particularly in carbaryl-treated tanks.

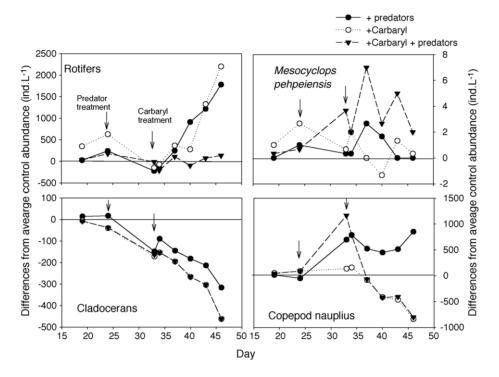


Fig. 5. Changes in average densities of rotifers, cladocerans, *M. pehpeiensis*, and copepod nauplii in the mesocosm tanks, expressed as differences from average density of control tanks with no predators and no carbaryl.

4. Discussion

One conspicuous impact of insecticide application on zooplankton communities is the increase in rotifer density (Day et al., 1987; Yasuno et al., 1988; Hanazato and Yasuno, 1990a; Van den Brink et al., 2002). Since rotifers are more tolerant than cladocerans to many pesticides including carbamate insecticide (carbaryl, Hanazato and Yasuno, 1990a), organophosphorus insecticide (azinphos-methyl, Sierszen and Lozano, 1998), organochlorine pesticide (lindane, Peither et al., 1996), and DCA (3,4-dichloroaniline, Jak et al., 1998), and at the same time, they are less competitive than cladocerans (Gilbert, 1988). Thus, the reduction of cladoceran populations due to insecticide application increases the rotifer population density.

In the present experiment, the addition of the predator *M. pehpeiensis* reduced cladoceran density and increased rotifer density in the tanks not treated with carbaryl (Fig. 4). The result indicates that predation by *M. pehpeiensis* affected cladocerans, but not rotifers. *M.*

pehpeiensis consumes small cladocerans and rotifers (Williamson, 1986; Chang and Hanazato, 2003a,b); our result suggests that M. pehpeiensis predation has a larger negative impact on cladocerans than on rotifers. The decrease of cladocerans by M. pehpeiensis predation might have allowed rotifer numbers to increase. However, the carbaryl application induced different patterns of change of rotifer densities between the tanks with high and low predator densities. In the tanks with low-predator density, the population density of rotifers increased after the carbaryl application, probably owing to the elimination of cladocerans by the carbaryl. However, in the tanks with high-predator density, the carbaryl treatment suppressed the growth of rotifer populations. Cyclopoid copepods are more tolerant to carbaryl and other insecticides than are cladocerans (Hanazato and Yasuno, 1990b; Havens, 1994; Van den Brink et al., 2002). The application of $0.5 \,\mathrm{mg}\,\mathrm{L}^{-1}$ carbaryl did not affect Mesocyclops (Fig. 2), which might have foraged on the rotifers after their preferred prey, the cladocerans, had been eliminated. Thus, it

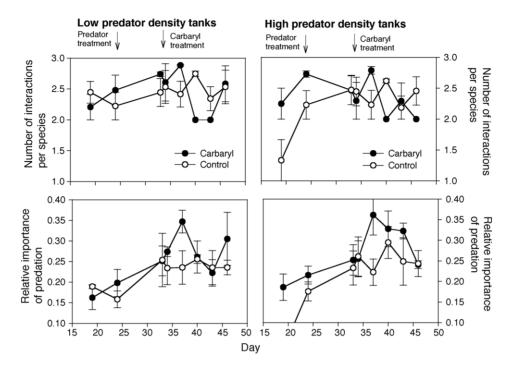


Fig. 6. Changes in the number of trophic interactions per species and the relative importance of predatory interactions in the food web in the mesocosm tanks.

seems that high predation pressure by *M. pehpeiensis* suppressed the increase of rotifers in the high-predator-density tanks with carbaryl treatment, where the superior competitors, the cladocerans, were eliminated. The increase of the relative importance of predatory interactions in the food web shown in these results also suggests that predation by *M. pehpeiensis* might play an important role in controlling rotifer populations after carbaryl application has reduced available prey items.

Since predators have a large impact on zooplankton community structure, the combined impact of predators and chemical application on zooplankton communities has been studied using mesocosms and artificial ponds (Peither et al., 1996; Hanazato, 1998b). Hanazato (1991) exposed different zooplankton communities (with and without the predator *Chaoborus*) to carbaryl and found that the *Chaoborus* altered the community response to the chemical by changing the community structure. Peither et al. (1996) found that the application of lindane changed a rotifer community indirectly through effects on predators. Loss of the predator *Chaoborus* due to lindane application was accompanied by an increase in the number of *Asplanchna*,

a predacious rotifer, and the predation by *Asplanchna* changed the community structure of the prey species. Cyclopoid copepods consume various zooplankton, including cladocerans and rotifers (Brandl, 1998). They are very common invertebrate predators in freshwater and are widely distributed. However, there is little information on combined effects of pesticides and copepods on zooplankton communities. The reason might be difficulties in controlling the copepods' density in experimental ponds and enclosures. Although we failed to completely eliminate copepods in the tanks, we succeeded in evaluating different copepod densities and in demonstrating their predation impact on the response of zooplankton to chemical disturbance.

In general, rotifers are known as less susceptible to chemical application than cladocerans. In our mesocosm experiment, the dominant rotifer species *Lepadella* sp. showed a general response to carbaryl application, the increase of its density after chemical application. However, although its density was less abundant in the tanks, *T. stylata* showed different response pattern. The density of *T. stylata* did not increase in the tanks with carbaryl treatment at both predator densities.

This result suggests that the response of rotifers to chemical application may differ depending on species, although their overall responses are determined by the chemical impact and biological interactions such as competition and predation.

Copepod adults seemed to be unaffected by carbaryl application, but their nauplii decreased and remained at low densities after carbaryl application regardless of the adult density. The reduction of nauplius density due to lindane application was reported in another mesocosm study (Peither et al., 1996). The results suggest that the copepods have ontogenetically different susceptibility to chemicals, as seen in the cladoceran Daphnia (Hanazato and Hirokawa, 2001). As mentioned above, copepods often play an important role as predators in ecosystems, and they have ontogenetically different trophic status in the food web as herbivorous nauplii and carnivorous older copepodids and adults (Williamson, 1986). Thus, their susceptibility to chemicals throughout their life cycle should be studied further to allow a better understanding of the response of copepod populations to chemicals and of the chemical impact on zooplankton communities through the activity of copepod populations.

Although the density of rotifers showed different patterns of change depending on the carbaryl treatment and the abundance of predators, the food web structure represented by the number of interactions showed no marked changes with carbaryl application. The number of interactions represents the number of trophic levels in a zooplankton community, and consequently shows the complexity of the food web (Sprules and Bowerman, 1988). The low impact of carbaryl on the food web structure of zooplankton in the present study seems to be due to the relatively simple trophic structure of the community established in the experiment, and to the fact that carbaryl mainly affected the cladocerans, which lie at an intermediate position in the food web. Cyclopoid copepods were only the predators included in the zooplankton community in the tanks, and were unaffected by carbaryl application. In addition, the role of the cladocerans eliminated by carbaryl application in the food web was taken over by rotifers. On the other hand, the relative importance of predatory interactions in the food web increased owing to the elimination of the cladocerans, the preferred food items of the predator as well as competitors of rotifers. Consequently, rotifers received increased predation pressure

from *M. pehpeiensis* and failed to develop high density, particularly in the high-predator-density tanks.

The results suggest that the impact of insecticides on zooplankton can be modified by the community structure of zooplankton. In particular, the abundance of predators can affect the succession of zooplankton that remain after the insecticide application. High abundance of predators that are not markedly affected by the chemicals can exert a serious impact on the remaining prey species and suppress their population increase, since predation pressure on the remaining prey might increase owing to elimination of alternative foods by the chemicals.

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